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Thiazolidinediones inhibit the transport activity of the mitochondrial pyruvate carrier proteins MPC-1 and MPC-2

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MPC-1 and MPC-2 were recently identified as essential components of the mitochondrial pyruvate transporter [1,2]. Here we report that thiazolidinediones (TZDs), which promote insulin sensitization in human skeletal muscle and increase the capacity to oxidize fatty acids, are acute inhibitors of pyruvate transport.

As PPAR-γ agonists, TZDs can also cause marked side effects that can be clinically prohibitive, such as plasma volume expansion, increased adiposity, congestive heart failure, and cardiovascular risk. Existing TZDs (rosiglitazone and troglitazone) and a prototype, PPAR-γ-sparing compound (MSDC-0160) selectively inhibit pyruvate oxidation in permeabilized rodent myocytes (primary and immortalized cultures) and patient-derived, permeabilized skeletal muscle myocytes.

Clinically relevant drug concentrations ($1\ \mu M < K_d < 9\ \mu M$) selectively inhibit pyruvate-driven respiration, but have no effect on oxidation of other complex I-linked substrates or succinate. Moreover, the respiratory inhibition can be rescued upon addition of methyl pyruvate, indicating that pyruvate dehydrogenase activity is unaffected.

Permeabilized C2C12 myoblasts show significantly compromised pyruvate-driven respiration upon shRNA knockdown of either MPC-1 or MPC-2. No respiratory defect is observed on a variety of other substrates, and the respiratory rate can be significantly restored with methyl pyruvate.

Furthermore, acute knockdown of either MPC-1 or MPC-2 left-shifts the dose–response curve for both TZDs and the highly specific MPC inhibitor UK5099, indicating that the MPC complex is indeed a target of modulation by TZDs. Experiments to determine the mechanism by which pyruvate transport relates to insulin-sensitization are underway.

In summary, these results provide two principal observations: (1) a rigorous, biochemical validation of MPC-1 and MPC-2 as obligatory components of the mitochondrial pyruvate transporter, and (2) the first demonstration that TZDs acutely inhibit pyruvate transport at clinically relevant concentrations.

References

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An electron dense substrate to study mitochondrial import sites *in situ*

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Mitochondria import a plethora of proteins into four distinct compartments, *via* complex and versatile networks of dynamic targeting pathways. Due to an intense biochemical effort our mechanistic knowledge has advanced significantly, yet there is a paucity of structural information and our understanding is incomplete. We aim to address this void by capture of an electron dense pre-protein through both mitochondrial TOM/TIM23 complexes concurrently. Electron cryo-tomography is being used to determine the three dimensional organisation of translocation sites *in situ*.

A novel translocation substrate has been designed, consisting of an N-terminal mitochondrial targeting presequence (pre-cytochrome b_2), followed by a section with the ability to fold and trap the protein in the membrane (dihydrofolate reductase) and a C-terminal electron dense tag to enable visualisation in the microscope (methallothionein). The substrate has been determined to be functional by *in vitro* transcription/translation methods with energised mitochondria and downstream protease protection. Formation of the TOM/TIM23 supercomplex has also been verified by native-PAGE. Subsequently, overexpression of the protein has been optimised in *Escherichia coli* and labeling procedures have been established in order to tag the protein with gold. To ensure that the tag can be visualised, electron cryo-tomography has been used to view the protein in solution. Mitochondria have been isolated from the yeast *Saccharomyces cerevisae* and tomograms were collected at a resolution to reveal internal molecular detail. Preliminary data is now being collected of mitochondria in the presence of the gold-labeled substrate. We aim to localise the import complexes on the mitochondrial surface and ultimately perform subtomogram averaging. By capture of a pre-protein in the act of import, fundamental structural information regarding the supramolecular organisation of the TOM/TIM supercomplex will be obtained.

In order to render mitochondrial import sites visible, an electron dense translocation substrate has been stalled across inner and outer membranes concurrently. This unique protein is both functional for import and arrest and can be seen in the microscope, demonstrating the value of such tools for *in situ* analysis of cellular mechanisms.

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Structure and conformational dynamics of the sodium/proline transporter PutP based on protein chemical and EPR spectroscopic analyses

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The sodium/proline symporter PutP is a member of the sodium/substrate symporter family (SSSF), which comprises more than 400 proteins of all three branches of the phylogenetic tree of life. PutP catalyzes the coupled translocation of sodium ions and proline with a stoichiometry of 1:1. It is employed by bacteria and archaea to accumulate proline as a nutrient or compatible solute under osmotic